Plant Physiol. (1995) 107: 385–391

Low Water Potential Disrupts Carbohydrate Metabolism in Maize (*Zea mays* L.) Ovaries[1]

Chris Zinselmeier, Mark E. Westgate*, Jeffrey R. Schussler, and Robert J. Jones

University of Minnesota, St. Paul, Minnesota (C.Z., R.J.J.); RECIRCA, Painesville, Ohio (J.R.S.); and United States Department of Agriculture-Agricultural Research Service, North Central Soil Conservation Research Laboratory, North Iowa Avenue, Morris, Minnesota 56267 (M.E.W.)

Water deficit during pollination increases the frequency of kernel abortion in maize (*Zea mays* L.). Much of the kernel loss is attributable to lack of current photosynthate, but a large number of kernels fail to develop on water-deficient plants even when assimilate supply is increased. We examined the possibility that assimilate utilization by developing ovaries might be impaired at low water potential (ψ_w). Plants were grown in the greenhouse in 20-L pots containing 22 kg of amended soil. Water was withheld on the first day silks emerged, and plants were hand-pollinated 4 d later when leaf ψ_w decreased to approximately −1.8 MPa and silk ψ_w was approximately −1.0 MPa. Plants were rehydrated 2 d after pollination. The brief water deficit inhibited ovary growth (dry matter accumulation) and decreased kernel number per ear by 60%, compared to controls. Inhibition of ovary growth was associated with a decrease in the level of reducing sugars, depletion of starch, a 75-fold increase in sucrose concentration (dry weight basis), and inhibition of acid invertase (EC 3.2.1.26) activity. These results indicate that water deficits during pollination disrupt carbohydrate metabolism in maize ovaries. They suggest that acid invertase activity is important for establishing and maintaining reproductive sink strength during pollination and early kernel development.

Water deficit during pollination increases the frequency of kernel abortion in maize (*Zea mays* L.) (Westgate and Boyer, 1986; Boyle et al., 1991; Schussler and Westgate, 1991). Much of the kernel loss is attributable to lack of current photosynthate (Boyle et al., 1991; Schussler and Westgate, 1991), but a large number of kernels fail to develop on water-deficient plants even when assimilate supply is increased culturally (Boyle et al., 1991; Schussler and Westgate, 1994) or genetically (Zinselmeier, 1991).

Low ψ_w inhibits dry matter accumulation and increases the concentration of assimilates in reproductive tissues (Zinselmeier, 1991; J.R. Schussler and M.E. Westgate, unpublished data). Endogenous levels of Suc in ovary, cob, and shank tissues increase in both amount and concentration. Such results imply that Suc partitioning to the reproductive stalk continues to some extent even at low ψ_w.

Under well-watered conditions, maize ovaries accumulate starch throughout pollination and early kernel growth (Reed and Singletary, 1989; Zinselmeier, 1991). Because partitioning into starch reserves depends on assimilate supply as well as demand (Jenner, 1982), assimilate supply may exceed demand during this critical period. At low ψ_w, starch levels in the reproductive shoot decrease (Zinselmeier, 1991), which suggests that assimilate supply is not sufficient to meet demand in the reproductive tissues of water-deficient plants.

Failure to metabolize Suc may lead to the depletion of starch reserves. During rapid kernel growth, Suc is unloaded passively from the phloem into the apoplast of the pedicel parenchyma (Shannon et al., 1986) and inverted to hexose sugars by a cell-wall-bound acid invertase (Shannon and Dougherty, 1972). The hydrolysis of Suc in the apoplast maintains a favorable gradient for continued unloading from the phloem and provides hexoses that are taken up by the basal endosperm cells (Shannon et al., 1986; Griffith et al., 1987). Hanft and Jones (1986) have shown that kernels induced to abort in vitro have only low levels of invertase activity in the pedicel. Also, maize mutants lacking pedicel invertase fail to produce normal kernels (Miller and Chourey, 1992). Suc import into developing sink tissues has been correlated with the activity of Suc synthase (Sung et al., 1988; Wang et al., 1993), and recent evidence suggests that Suc synthase activity may be modulated by tissue carbohydrate status (Koch et al., 1992). How a water deficit might affect the activity of these Suc-metabolizing enzymes in maize ovaries is not known, but inhibition of invertase or Suc synthase activity could lead to a cessation of ovary growth even if assimilates were available.

We examined the in vitro activities of soluble and insoluble acid invertases and Suc synthase extracted from ovaries of well-watered and water-deficient plants. Our objec-

[1] Supported in part by U.S. Department of Agriculture Cooperative Grant 86-CRCR-1-2055 to M.E.W. Scientific Journal Series No. 21,153. Contribution from U.S. Department of Agriculture-Agricultural Research Service in cooperation with the University of Minnesota Agricultural Experiment Station. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap. Product names are provided for the benefit of the reader and do not imply endorsement or preferential treatment by the U.S. Department of Agriculture or the University of Minnesota.

* Corresponding author; e-mail westgate@soils.mrsars.usda.gov; fax 1-612-589-3787.

Abbreviations: DAP, day(s) after pollination; ψ_w, water potential.

tive was to determine whether inhibition of these enzymes at low ovary ψ_w could account for Suc accumulation in ovaries and the failure of ovaries to develop in water-deficient plants.

MATERIALS AND METHODS

Plant Culture

Maize (*Zea mays* L.) hybrid A619 × W64a was grown in a greenhouse under natural sunlight supplemented for 14 h with 1000-W high-intensity discharge metal halide lamps (minimum PAR 700 μE m^{-2} s^{-1} at the uppermost leaf) at 30/20 ± 1°C day/night temperature and 50/70 ± 10% RH. Seeds were sown in 20-L pots containing 22 kg of 2:1 (v/v) sand:soil mix and fertilized according to the procedure of Schussler and Westgate (1991). Plants were arranged in 71-cm rows with 30 cm between plants (approximately 46,000 plants ha^{-1}). When plants were sampled, remaining plants were rearranged to maintain this population density.

Water-Deficit Treatment

The low ψ_w treatment was imposed to completely inhibit photosynthesis during flowering and early kernel growth. Water was withheld from randomly selected plants beginning on the day silks first emerged. Plants were hand-pollinated between 8 and 10 AM 4 d later when silk ψ_w was approximately −1.0 MPa. Otherwise, ear shoots were covered to prevent pollination. Plants were rewatered 2 DAP and remained well watered until harvest.

ψ_w Measurements

Ear leaf and silk ψ_w were measured by isopiestic thermocouple psychrometry at 25°C and corrected for heat of respiration (Westgate and Boyer, 1986). Leaf discs (3.1 cm^2) were sampled between 8 and 10 AM from the central portion of the ear leaf blade and immediately sealed in a psychrometer cup coated with petrolatum to minimize sorption effects. Just prior to pollination, 20 to 50 exposed silks (1.5 cm) were cut approximately 4 cm distal to the tip of the ear and immediately sealed in a prepared psychrometer cup. Equilibration time was typically 2 to 3 h. Since silks of well-watered plants were elongating, a decrease in silk turgor (and ψ_w) could have occurred during ψ_w measurement. Previous work (Westgate and Boyer, 1985) indicated that turgor loss could be as much as 0.2 MPa. No loss of turgor should have occurred in silks sampled from water-deficient plants, since silk growth is completely inhibited at $\psi_w \leq -0.8$ MPa (Westgate and Boyer, 1985).

Leaf Photosynthesis

Photosynthesis rates of fully exposed leaves in the upper canopy were measured on clear days between 12 and 3 PM using a Li-Cor 6200 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE). Rates of CO_2 depletion were measured in a 1-L chamber with flow rate through the desiccant adjusted to maintain RH at 45 ± 2%. Leaf area exposed within the chamber was 22.5 cm^2. During the 30-s measurement period, CO_2 concentration (initially 340–380 μbar) was typically depleted approximately 60 μbar for well-watered plants.

Carbohydrate Analysis

Plants were sampled for dry matter accumulation, carbohydrate analysis, and enzyme activity on −4 (control only), −2, 0, 1, 3, 8, and 13 DAP and divided into vegetative (leaf and stem) and reproductive (kernel, cob, and husk) components. Kernels (or ovaries prior to fertilization) were sampled intact from the middle of the rachis and contained embryo plus endosperm (or egg sac plus nucellus, as appropriate), pericarp, and pedicel tissue. All kernels sampled 1, 3, 8, and 13 DAP were pollinated. It was not possible to distinguish between aborting and developing kernels on water-deficient plants at 0, 1, or 3 DAP. Kernel data presented for these dates are for a population having approximately 50% abortion (Fig. 2). Plant parts were heated at 90°C for 1 h and dried at 60°C for 72 to 96 h to obtain dry weights. Subsamples taken for carbohydrate analysis were immediately frozen on dry ice, lyophilized, and ground to pass a 20-mesh screen. Soluble sugars (Glc plus Fru plus Suc) were determined by HPLC. Starch was measured spectrophotometrically (Hanft and Jones, 1986).

Invertase and Suc Synthase Activity

Plant samples taken for enzyme analysis were frozen immediately on dry ice and stored at −20°C. Ovary/kernel tissue (200–400 mg of frozen tissue) was homogenized in 3 mL of 50 mM Hepes-NaOH buffer (pH 7.0) containing 10 mM $MgCl_2$, 1 mM EDTA, 10 mM ascorbate, and 2.5 mM DTT. The slurry was centrifuged and the supernatant desalted through Sephadex G-25 (superfine) (Pharmacia Fine Chemical, Piscataway, NJ) according to the method of Helmerhorst and Stokes (1980). The pellet was washed three times with 5 mM Hepes-NaOH buffer (pH 7.4) to remove endogenous sugars and then resuspended.

Soluble acid invertase (EC 3.2.1.26) activity was assayed according to the method of Giaquinta et al. (1983) with the following modifications. A 50- to 150-μL aliquot of the desalted extract was incubated with 10 mM Suc in 50 mM Na-acetate buffer (pH 4.8) containing 15 mM $MgCl_2$ (1 mL). The sample was incubated at 30°C for 30 min, and the reaction was terminated by adding 1 mL of alkaline copper reagent (Nelson No. 1). Suc cleavage was determined by the Nelson-Somogyi reducing sugar assay (Nelson, 1944) using D-Glc as the standard.

Insoluble acid invertase was assayed as described by Giaquinta et al. (1983) with the following modifications. After the sample was washed three times in 5 mM Hepes-NaOH buffer (pH 7.4), the insoluble cell wall pellet was resuspended, and a 50- to 150-μL aliquot was incubated with 10 mM Suc in 50 mM Na-acetate buffer (pH 4.8) containing 15 mM $MgCl_2$ (1 mL). Incubation and quantification of reducing sugars were as described for soluble acid invertase.

Suc synthase (EC 2.4.1.13) was assayed in the cleavage direction according to the method of Sowokinos et al. (1985) with the following modifications. A 100- to 200-μL aliquot of the desalted extract was incubated with 250 mM Suc in 50 mM Hepes-NaOH buffer (pH 7.4) containing 10 mM UDP and 15 mM $MgCl_2$ (1 mL) at 30°C for 30 min. The reaction was terminated by adding 500 μL of 1 N NaOH. The Fru produced was quantified by the Nelson-Somogy method (Nelson, 1944). Suc synthase was assayed in the synthesis direction as described by Claussen et al. (1985) with the following modifications. A 100- to 275-μL aliquot of the desalted extract was incubated with 50 mM Hepes-NaOH (pH 7.4) containing 15 mM $MgCl_2$, 20 mM Fru, and 10 mM UDP-Glc (1 mL). Samples were incubated as in the cleavage reaction. Suc was quantified according to the method of Roe (1934).

Protein content in the desalted extract was determined using the bicinchoninic acid assay (Smith et al., 1985) as modified by R. Lovrein (personal communication). Reagent A (0.1 M NaOH, 0.1 M $NaHCO_3$, 0.1 M Na_2CO_3, 1% [w/v] sodium potassium tartrate tetrahydrate [Rochelle salts, Aldrich Chemical Co., Milwaukee, WI], and 0.01% [w/v] bicinchoninic acid disodium salt) and reagent B (4% [w/v] $CuSO_4 \cdot 5H_2O$) were mixed 49:1 (v/v) immediately prior to use. A 50- to 200-μL aliquot of enzyme extract was diluted to 1 mL with assay buffer, mixed with 2 mL of reagent (A plus B), and incubated at 37°C for 30 min. Protein level was measured as A_{562} against a standard curve of BSA.

RESULTS

Water-Deficit Treatment

In well-watered plants, leaf ψ_w was −0.4 to −0.6 MPa from silk emergence to 13 DAP (Fig. 1). When water was withheld at the time of silk emergence, leaf ψ_w decreased to about −1.8 MPa within 4 d. Leaf photosynthesis decreased with leaf ψ_w from approximately 26 to 0.4 μmol m^{-2} s^{-2} on the day of pollination. Both leaf ψ_w and leaf photosynthesis recovered to control values soon after plants were rewatered 2 DAP. Silk ψ_w also decreased in response to the water deficit from −0.4 MPa at the time of silk emergence to −1.0 MPa on the day of pollination (Fig. 2). This brief water deficit decreased kernel number from approximately 775 per ear in the controls to approximately 350 per ear in water-deficient plants. Water-deficient plants had fewer silks exposed for pollination than did well-watered plants, but the difference between treatment means was not statistically significant (data not shown). Thus, kernel loss in the water-deficient plants was due primarily to zygotic abortion (Westgate and Boyer, 1986; Bassetti and Westgate, 1993). The decrease in kernel number and changes in plant water status are similar to those reported for field-grown plants (Schussler and Westgate, 1994).

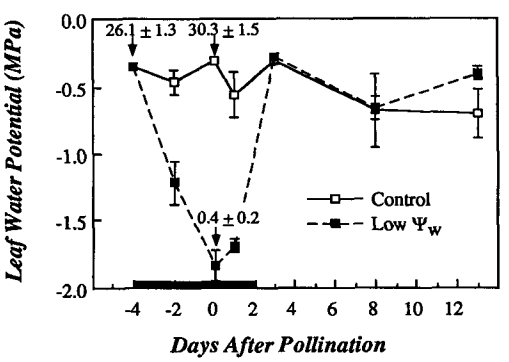

Figure 1. Leaf ψ_w of control (□) and water-deficient (■) maize plants during pollination and early kernel growth. Photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) were measured on days marked by arrows. Horizontal bar indicates when water was withheld. Data are the means ± SE of three or four plants.

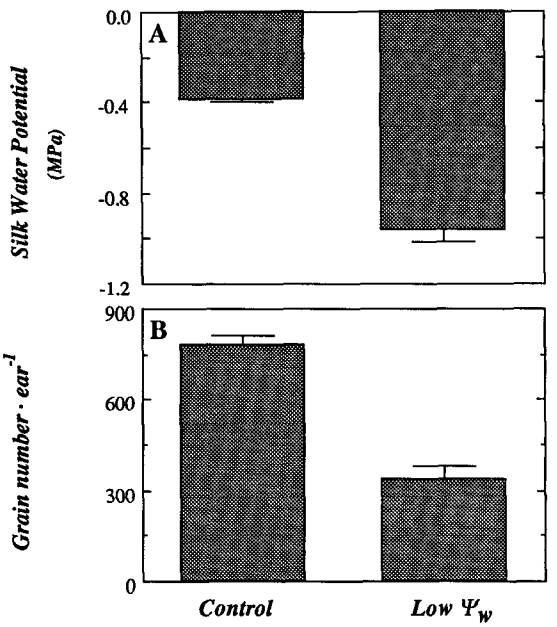

Figure 2. Silk ψ_w at the time of pollination (A) and grain number per ear (B) for control and water-deficient plants. Water was withheld at the time of silk emergence and plants were pollinated by hand 4 d later. Control plants remained well watered throughout and were also pollinated 4 d after first silk emergence. Data are the means ± SE of 18 plants for silk ψ_w and 8 plants for grain number.

Ovary and, subsequently, kernel dry weight increased at about 0.4 mg d^{-1} from the time of silk emergence to 3 DAP (Fig. 3) and then rapidly thereafter as kernels began the linear phase of growth. The water deficit inhibited the rate of dry matter accumulation so that, by the day of pollination, ovaries on water-deficient plants were arrested in development. Upon rewatering at 2 DAP, aborted kernels accumulated little dry matter, whereas setting kernels resumed growth after a 1- to 2-d lag and accumulated dry matter at a rate comparable to the controls (Fig. 3).

Ovary/Kernel Carbohydrate Status

Reducing sugar (Glc plus Fru) content increased from the time of silk emergence through pollination and early kernel development (Fig. 4A). On a dry weight basis, the level of reducing sugars peaked at the time of pollination and declined thereafter (Fig. 4B). Values were similar to those reported for other maize genotypes (Reed and Singletary,

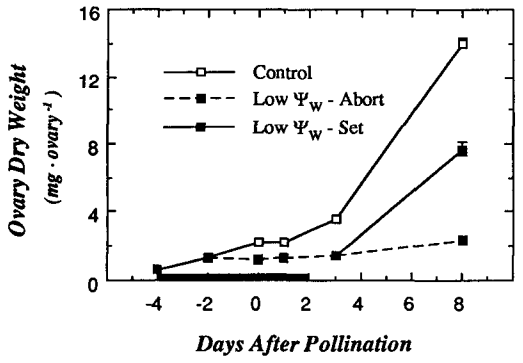

Figure 3. Dry weight of ovaries sampled from plants pollinated at high silk ψ_w (□) or low silk ψ_w (■). Aborting kernels (Abort) on water-deficient plants could be distinguished from setting kernels (Set) by 8 DAP. Horizontal bar indicates when water was withheld. Data are the means ± SE of three to four plants.

1989). In water-deficient plants, reducing sugars followed a pattern similar to that of kernel dry weight (Fig. 3). Sugar accumulation ceased at low ψ_w and failed to resume upon rewatering in aborting kernels. Setting kernels accumulated reducing sugars after a 1- to 2-d lag at a rate similar to the controls (Fig. 4, A and B).

In the controls, Suc content and level decreased rapidly during silk emergence to a minimum at the time of pollination and then increased as kernels began to develop (Fig. 4, C and D). A rapid decrease in kernel Suc at approximately the time of pollination also was observed by Reed and Singletary (1989) and Schussler and Westgate (1991). In water-deficient plants, ovary Suc increased rapidly between −2 and 0 DAP. On a dry weight basis, the level of Suc reached a maximum approximately 75-fold greater than in the controls on the day of pollination. When water-deficient plants were rewatered, Suc decreased in aborting kernels but recovered to control values in setting kernels by 13 DAP.

Controls contained about 100 μg of starch at the time of silk emergence and accumulated starch slowly during pollination and the lag phase of kernel development (Fig. 4E). Starch concentration (per g dry weight) declined to a minimum 8 DAP as kernel dry matter increased. In water-deficient plants, ovaries depleted starch reserves between the time of silk emergence and pollination. Upon rewatering, aborting kernels accumulated starch temporarily, but levels decreased after 8 DAP. Setting kernels accumulated starch at the control rate.

Acid Invertase and Suc Synthase Activity

The differences in Suc and reducing sugar levels in ovaries of control and water-deficient plants (Fig. 4) suggested that one or more enzymes involved in Suc metabolism might be inhibited at low ovary ψ_w. We measured the in vitro activities of the soluble and insoluble forms of acid invertase, neutral invertase, and Suc synthase. Preliminary measurements indicated that the acid invertases were the dominant Suc-metabolizing enzymes during pollination and early kernel growth. Neutral invertase was detected at low levels, but activity did not vary in response to low ψ_w (Zinselmeier, 1991). Also, Suc synthase activity was not detectable until 13 DAP. Therefore, developmental profiles

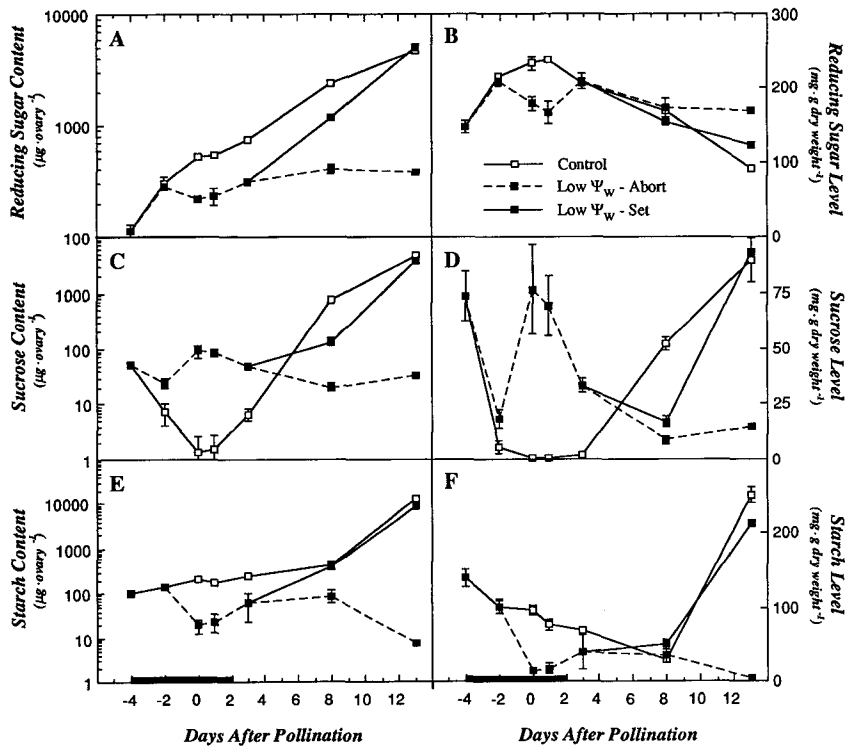

Figure 4. Reducing sugar (A and B), Suc (C and D), and starch (E and F) levels in ovaries sampled from maize plants pollinated at high silk ψ_w (□) or low silk ψ_w (■). Data are expressed on a per-ovary basis (left) and on a per-gram dry weight basis (right). Aborting kernels (Abort) on water-deficient plants were distinguished from setting kernels (Set) at 8 and 13 DAP. Horizontal bar indicates when water was withheld. Data are the means ± SE of three or four plants.

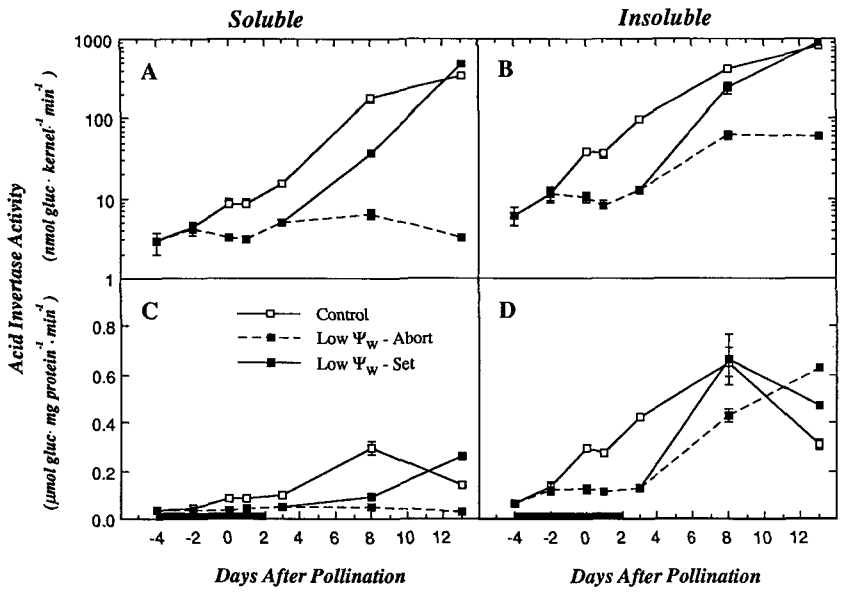

Figure 5. Soluble (A and C) and insoluble (B and D) acid invertase activity extracted in ovaries sampled from maize plants pollinated at high silk ψ_w (□) or low silk ψ_w (■). Data are expressed on a per-kernel basis (A and B) and per-milligram of protein assayed basis (C and D). Aborting kernels (Abort) on water-deficient plants were distinguished from setting kernels (Set) at 8 and 13 DAP. Horizontal bar indicates when water was withheld. Data are the means ± SE of three or four plants.

are presented only for the soluble and insoluble acid invertases.

The activities of both forms of acid invertase followed similar patterns during flowering and early kernel growth (Fig. 5). In the controls, total enzyme activity was low at the time of silk emergence but increased logarithmically during pollination and the lag phase of development. Invertase activity per milligram of protein peaked 8 DAP. The subsequent decrease in activity was coincident with the rapid increase in kernel protein content (data not shown) and initiation of rapid starch synthesis (Fig. 4).

The water deficit inhibited the activity of both the soluble and insoluble acid invertases compared to controls (Fig. 5). In aborting kernels, soluble activity remained at the low levels observed at the time of silk emergence and failed to recover upon rewatering (Fig. 5, A and C). Soluble activity in setting kernels increased upon rewatering at a rate similar to the controls but with a 4- to 5-d lag. Insoluble acid invertase activity also remained low until plants were rewatered (Fig. 5B). In aborting kernels, activity recovered slowly, but the rate was similar to that of the controls when expressed per milligram of protein assayed (Fig. 5D). Insoluble activity in setting kernels recovered to control levels by 8 DAP.

Suc synthase activity was not detected in maize ovaries/kernels until 13 DAP (Fig. 6). The increase in enzyme activity at that time coincided with the onset of rapid starch deposition (Fig. 4), in agreement with results of Doehlert et al. (1987). A low level of activity (cleavage direction only) was detected in aborted kernels from water-deficient plants even though these kernels failed to accumulate starch after pollination (Fig. 4). Suc synthase activity in setting kernels on these same plants was similar to the control (Fig. 6).

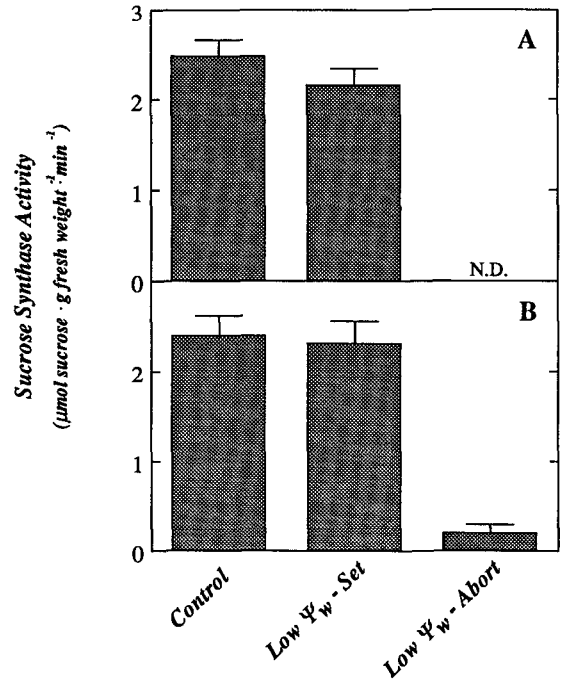

Figure 6. Suc synthase activity in maize ovaries sampled 13 DAP. Activity is shown for both synthesis (A) and cleavage (B) directions. Suc synthase activity was not detectable in ovaries or kernels from the time of silk emergence to 8 DAP. Water was withheld from "Low ψ_w" plants from the time of silk emergence until 2 DAP. Aborted (Abort) and setting (Set) ovaries were sampled from the same region of the ear. Data are the means ± SE of three plants. N.D., Not detected.

DISCUSSION

More than half of the ovaries pollinated at low ψ_w failed to set kernels (Fig. 2). Since low ovary ψ_w does not prevent

pollen germination, pollen tube growth, or fertilization (Westgate and Boyer, 1986; Bassetti and Westgate, 1993), kernel loss on the water-deficient plants must have resulted from zygotic abortion. In contrast to ovaries on control plants, water-deficient ovaries depleted pools of reducing sugars and starch while maintaining a high level of Suc (Fig. 4). These results suggest that reproductive growth ceased at low ψ_w because the normal flux of reduced carbon from the translocation stream to sites of metabolism within the ovaries was disrupted.

Large changes in the amount and concentration (per gram dry wt) of sugars occurred in control ovaries between the time of silk emergence and pollination (Fig. 4). Suc concentration, in particular, decreased rapidly to very low levels, which would favor continued unloading from the phloem into the pedicel parenchyma (Shannon et al., 1986). As such, the rapid decrease in Suc concentration may be a critical step in establishing the newly formed zygote as a sink for assimilates.

In water-deficient plants, the ovary Suc level increased as the water deficit developed and was approximately 75-fold greater than the control level by the time of pollination (Fig. 4). The accumulation of Suc at low ovary ψ_w is consistent with previous reports (Schussler and Westgate, 1991, 1994) but was particularly dramatic in this study because of the rapid and extensive depletion of Suc in control ovaries (Fig. 4). The fact that the increase in Suc coincided with the cessation of dry matter accumulation (−4 and −2 DAP, Fig. 3) implies that demand for Suc in the ovary decreased in advance of a change (decrease) in assimilate unloading in the pedicel. The complete reversal from depletion to accumulation of Suc during this period suggests that conditions for zygotic abortion may have been established within the ovaries even before fertilization occurred. This possibility is supported by the findings of Westgate and Boyer (1986), who observed that kernel number per ear did not recover on water-deficient plants that had been rewatered prior to pollination.

The starch content of control ovaries increased steadily, albeit slowly, during silk emergence and pollination (Fig. 4), but water-deficient ovaries depleted starch to approximately 10% of control levels by the day of pollination. This loss of starch is similar to that reported for soybean ovules after fertilization (Peterson et al., 1990) when sink intensity is low (Brun and Betts, 1984), which suggests that starch is a source of reserve carbohydrate for the newly formed zygote. The depletion of this reserve in the maize ovary is enigmatic considering the rapid increase in Suc content and concentration that occurred at the same time (Fig. 4). Coupled with the decrease in reducing sugars, the rapid depletion of starch implies that the embryo could not utilize this source of carbon. It is possible that Suc unloaded from the phloem did not reach the embryo, i.e. accumulated in the pedicel, or could not be metabolized. As discussed below, our measurements of Suc-metabolizing enzymes suggest that both problems may have developed at low ψ_w.

Acid invertases were the dominant Suc-metabolizing enzymes in ovaries during silk emergence and pollination (Fig. 5). Suc synthase activity did not develop until rapid starch deposition began at about 13 DAP (Fig. 6). The activities of both soluble and insoluble forms of acid invertase were inhibited at low ovary ψ_w, compared to controls. The inhibition of either of these enzymes could lead to an accumulation of Suc, provided phloem unloading continued. Since the carbohydrate data collected in this study represent an average for the entire ovary, it is not possible to conclude with certainty where Suc accumulated within the ovary. However, Westgate et al. (1991) reported that the amount of Suc in the apoplast of water-deficient ovaries was 35-fold greater than in the controls. In their study, ovary free space was infiltrated under isosmotic conditions to minimize solute leakage from the symplasm. Also, solute concentration was corrected for apoplast volume and symplasm exchange. Using their estimate of 3.5% apoplast volume (Westgate et al., 1991) and the Suc data in Figure 4, we calculate that the amount of Suc in the symplasm of water-deficient ovaries was about 50-fold greater than in the controls on the day of pollination. Since the ovary is predominately pedicel tissue at this time (Westgate and Boyer, 1986), it is reasonable to conclude that the difference in Suc levels between control and water-deficient ovaries at pollination largely reflected conditions within the pedicel. If so, the data suggest that Suc continued to unload from the phloem at low ovary ψ_w but accumulated in the symplasm and apoplasm of the pedicel because of low invertase activity. This conclusion is supported by the findings of Miller and Chourey (1992), who showed that developmental failure of *miniature-1* kernels of maize was linked to lack of invertase activity in the pedicel tissue during the early stages of kernel development. It would be interesting to examine whether *miniature-1* kernels can develop under drought conditions.

These results support earlier work showing that invertase plays an important role in establishing and/or maintaining reproductive sink strength in maize (Hanft and Jones, 1986; Doehlert and Felker, 1987; Miller and Chourey, 1992). Although our data do not exclude the possibility that Suc enters the ovary without hydrolysis (Griffith et al., 1987; Schmalstig and Hitz, 1987), the increase in Suc concentration in the pedicel region and lack of effect of low ψ_w on neutral invertase activity within the ovary (Zinselmeier, 1991) suggest that reduced carbon enters the ovary primarily as reducing sugars. Regardless of the actual path, the data imply that failure to utilize available Suc at low ψ_w may ultimately lead to ovary abortion.

Inhibition of acid invertase activity at low ovary ψ_w decreases the apparent demand for assimilates at a time when reproductive sink strength already is low (Edmeades and Daynard, 1979; Brun and Betts, 1984; Schussler and Westgate, 1991). Hydrolysis of Suc by wall-bound invertase is thought to maintain a favorable Suc gradient and cell turgor for rapid phloem unloading (Shannon et al., 1986). Accumulation of Suc in the pedicel at low ψ_w may explain why it has not been possible to improve seed set in water-deficient plants by increasing the amount of carbohydrate reserves prior to silk emergence (Schussler and Westgate, 1994; Zinselmeier et al., 1994). The stem of maize

is a major sink for assimilates during anthesis (Edmeades and Daynard, 1979; Setter and Meller, 1984; Zinselmeier, 1991) and remains a strong sink even at low ψ_w (Schussler and Westgate, 1991, 1994; Zinselmeier, 1991). Boyle et al. (1991) have shown that it is possible to overcome the lack of assimilate movement to the ovaries and recover approximately 70% of the kernel loss at low ψ_w by infusing a large amount of Suc (>4 g d^{-1}) into the stem of water-deficient plants prior to pollination. This approach likely overwhelms the translocation stream with Suc so that low ovary sink strength is less of a disadvantage.

Whether accomplished artificially (Boyle et al., 1991; Mosjidis et al., 1993), culturally (Zinselmeier, 1991; Schussler and Westgate, 1994), or genetically (Edmeades et al., 1993), improvements in seed set under adverse environmental conditions have been closely coupled to the maintenance of ovary growth. Because lack of invertase activity may limit carbon flux required for ovary growth, understanding the physiological basis for the loss of activity in water-deficient plants could be key to improving seed set at low ψ_w. We have observed that water deficits alter the pH, ion concentration, and osmotic potential of the ovary apoplasm (Westgate et al., 1991). However, these changes appear to be too small to account for the observed inhibition of invertase activity. Whether decreased synthesis of invertase protein or efficiency of substrate utilization could account for the loss of activity is the subject of current studies.

Received May 23, 1994; accepted October 24, 1994.
Copyright Clearance Center: 0032-0889/95/107/0385/07.

LITERATURE CITED

Bassetti P, Westgate ME (1993) Water deficit affects receptivity of maize silks. Crop Sci **33:** 279–282

Boyle MG, Boyer JS, Morgan PW (1991) Stem infusion of liquid culture medium prevents reproductive failure of maize at low water potentials. Crop Sci **31:** 1246–1252

Brun WA, Betts KJ (1984) Source/sink relations of abscising and nonabscising soybean flowers. Plant Physiol **75:** 187–191

Claussen W, Loveys BR, Hawker JS (1985) Comparative investigations on the distribution of sucrose synthase activity and invertase activity within growing, mature and old leaves of some C_3 and C_4 plant species. Physiol Plant **65:** 275–280

Doehlert DC, Felker RC (1987) Characterization and distribution of invertase activity in developing maize (*Zea mays*) kernels. Physiol Plant **70:** 51–57

Edmeades GO, Bolaños J, Hernández M, Bello S (1993) Causes for silk delay in a lowland tropical maize population. Crop Sci **33:** 1029–1035

Edmeades GO, Daynard TB (1979) The relationship between final yield and photosynthesis at flowering in individual maize plants. Can J Plant Sci **59:** 585–601

Giaquinta RT, Lin W, Sadler NL, Franceschi VR (1983) Pathway of phloem unloading of sucrose in corn roots. Plant Physiol **72:** 362–267

Griffith SM, Jones RJ, Brenner ML (1987) *In vitro* sugar transport in *Zea mays* L. kernels. Plant Physiol **84:** 472–475

Hanft JM, Jones RJ (1986) Kernel abortion in maize (*Zea mays* L.). I. Carbohydrate concentration patterns and acid invertase activity of maize kernels induced to abort *in vitro*. Plant Physiol **81:** 503–510

Helmerhorst E, Stokes GB (1980) Microcentrifuge desalting: a rapid, quantitative method for desalting small amounts of protein. Anal Biochem **104:** 130–135

Jenner CF (1982) Storage of starch. *In* FA Loewus, W Turner, eds, Plant Carbohydrates. I. Intracellular carbohydrates. Encyclopedia of Plant Physiology, New Series, Vol 13A. Springer-Verlag, New York, pp 700–747

Koch KE, Nolte KD, Duke ER, McCarty DR, Avigne WT (1992) Sugar levels modulate differential expression of maize sucrose synthase genes. Plant Cell **4:** 59–69

Miller ME, Chourey PS (1992) The maize invertase-deficient *miniature-1* seed mutation is associated with aberrant pedicel and endosperm development. Plant Cell **4:** 297–305

Mosjidis CO'H, Peterson CM, Truelove B, Dute RR (1993) Stimulation of pod and ovule growth of soybean, *Glycine max* L Merr, by 6-benzylaminopurine. Ann Bot **71:** 193–199

Nelson N (1944) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem **153:** 375–380

Peterson CM, Williams JC, Kuang A (1990) Increased pod set of determinate cultivars of soybean, *Glycine max*, by 6-benzylaminopurine. Bot Gaz **151:** 322–330

Reed AJ, Singletary GW (1989) Roles of carbohydrate supply and phytohormones in maize kernel abortion. Plant Physiol **91:** 986–992

Roe JH (1934) A colorimetric method for the determination of fructose in blood and urine. J Biol Chem **107:** 15–22

Schmalstig JG, Hitz WD (1987) Transport and metabolism of a sucrose analog (1'-fluorosucrose) into *Zea mays* L. endosperm without invertase hydrolysis. Plant Physiol **85:** 902–905

Schussler JR, Westgate ME (1991) Kernel set of maize at low water potential. II. Sensitivity to reduced assimilate supply at pollination. Crop Sci **31:** 1196–1203

Schussler JR, Westgate ME (1994) Increasing assimilate reserves does not prevent kernel abortion at low water potential in maize. Crop Sci **34:** 1569–1576

Setter TL, Meller VH (1984) Reserve carbohydrate in maize stem. [^{14}C]Glucose and [^{14}C]sucrose uptake characteristics. Plant Physiol **75:** 617–622

Shannon JC, Dougherty CT (1972) Movement of ^{14}C-labeled assimilates into kernels of *Zea mays* L. II. Invertase activity of the pedicel and plancento-chalazal tissues. Plant Physiol **49:** 203–206

Shannon JC, Porter GA, Knievel DP (1986) Phloem unloading and transfer of sugars into developing corn endosperm. *In* J Cronshaw, WJ Lucas, RT Giaquinta, eds, Phloem Transport. Alan R Liss, New York, pp 197–209

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem **150:** 76–85

Sowokinos JR, Lulai EC, Knoper JA (1985) Translucent tissue defects in *Solanum tuberosum* L. I. Alterations in amyloplast membrane integrity, enzyme activities, sugars and starch content. Plant Physiol **89:** 1066–1070

Sung SJ, Xu CP, Black CC (1988) Identification of actively filling sucrose sinks. Plant Physiol **89:** 1117–1121

Wang F, Sanz A, Brenner ML, Smith A (1993) Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol **101:** 321–327

Westgate ME, Boyer JS (1985) Osmotic adjustment and the inhibition of leaf, root, stem, and silk growth at low water potentials in maize. Planta **164:** 540–549

Westgate ME, Boyer JS (1986) Reproduction at low silk and pollen water potentials in maize. Crop Sci **26:** 951–956

Westgate ME, Hampton RE, Jones RJ (1991) Influence of water deficit on acid invertase activity and apoplast environment in maize ovaries. *In* Agronomy Abstracts. American Society of Agronomy, Madison, WI, p 136

Zinselmeier C (1991) The role of assimilate partitioning and metabolism in maize kernel development at low water potential (dissertation abstract No. DA9212104). PhD thesis, University of Minnesota, St. Paul

Zinselmeier C, Westgate ME, Jones, RJ (1994) Kernel set does not vary with source/sink ratio in maize. Crop Sci **35:** (in press)